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Progress Report on the Grant NGR-09-134-001 entitled:

"THE BIOCHEMISTRY AND BIOPHYSICS OF STRESS OF CONNECTIVE TISSUE"

Introduction

This grant supported program is essentially divided into two phases; the one concerned with the metabolism of skin collagen in stressed animals and the other concerned with the biophysics of the skin of stressed animals.

A) Metabolism

C^{14} labeled proline was given to young rats and these animals were stored for up to 2 months subsequent to injection. After this period of time the great bulk of C^{14} found in these animals was contained in skin collagen as either proline or hydroxyproline. These animals were then fasted overnight and received a physiologic dose of stress hormone (cortisol). The animals were stored in a cage which restricted motion and permitted the collection of expired CO_2. These animals all expired 50% more radioactive CO_2 subsequent to cortisol administration than they did prior to stress hormone. This release of increased amounts of $C^{14}O_2$ occurred 4-6 hours after injection. After this period, the animals were sacrificed and their liver glycogen isolated. The liver glycogen of stressed rats was considerably more radioactive than that of the unstressed control animals. These results suggest that the catabolism of skin collagen induced by stress hormone released free amino acids which are used to make liver glycogen and thus provide some of the energy for the "flight or fight reaction".

B) Biophysics

Skin may be considered on a model basis from a gross consideration as a very amorphous medium containing crystylites of collagen (ordered segments) dispersed in an amorphous matrix. Due to this character, skin would be expected to give a complex collection of phenomenological behaviors which would occur simultaneously under various enviromental conditions. From preliminary

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experiments on rat skin, this has been found to be the case. Thus it has become imperative to isolate and resolve the individual macroscopic phenomena which occur in skin simultaneously under various physical conditions; these conditions are mechanical stress, elongation, temperature and time conditions. Specialised equipment has been designed and built toward this end. Due to the unique macroscopic characteristics of skin, we have found that little or no commercial equipment that is available for mechanical testing can be directly used for testing of skin for the purpose of isolating these individual macrospopic phenomena so that they can be interpreted in terms of macroscopic thermodynamic and kinetic theory. These theoretical considerations should be capable of being related to the molecular structural changes occurring in skin under various biochemical, pharmacological, physiological and pathological conditions. This does not mean to say that commercial equipment has not been used to test skin, it only means to say that it has not achieved successful separation without extensive modification of the fundamental variables of the equipment. For example, commercial equiment has been used to approximate the tensile strength of skin of various animals and under various physical and biological conditions. However, tensile strength or any other strength measurement has never been successfully analysed from a theoretical point of view using the laws of thermodynamics or kinetics or statistical mechanics. Even on single crystals of pure elements using cohesive energy calculations, the experimental values are often different from the theoretical values by orders of magnitudes, thus strength of a material whatever it may be, metal, polymer or other, is still best determined experimentally and can only be related to molecular structural change by drawing parallels; for example, in skin, the tensile strength increase sometimes parallels the increasedin skin collagen concentration with the age of the animal.

Equipment for measuring specific volume changes in skin has been built by us since specific volume is a fundamental parameter in thermodynamics. The density and the specific volume of skin can be determined by this equipment over a temperature range from about 0°C to about 80°C. This study is limited in the high temperature range due to the generation of the skin specimen which begins to become visually apparent around 55°C depending on the time of exposure of the skin specimen. The density of a 500gm rat stomach skin has been found to be 1.085gm/ccs at 30°C. Also, the coefficient of volumetric expansion of this rat skin was determined over the physiological temperature range and was found to be $3.3\times10^{-4}\,{}^{o}C^{-1}$. These tests were conducted for the purpose of evaluating the sensitivity and operation of the equipment and to obtain a general idea of the volumetric characteristics of skin and the behavior of skin under these test conditions such as the fluid extrusion affect observed in the 50°C temperature range and above. Both the specific volume and the coefficient of volumetric expansion of skin are needed for any application of thermodynamics to the mechanical behavior governed by state dependent laws. Also the density of the specimen must be known for any mechanical stress experiment conducted on the specimen. However, even though the present equipment will determine the specific volume of skin over the above temperature range, it has been found not to be sensitive enough to detect the first-order phase transition in skin from collagen to gelatin occurring at higher temperature, for example around 65°C. Therefore, more sensitive equipment must be designed if this first order thermodynamic phenomena is to be observed in skin by volumetric measurements.

The second piece of equipment that has been developed is a stress or force relation-temperature device with which force-temperature and force-time changes can be observed in skin. This piece of equipment has been used in testing of skin and it has been found that in the physiological temperature range (30-40° temperature range) that the dominant phenomena in skin that govern its mechanical behavior are hydrodynamics (or kinetic) rather than thermodynamics. However, thermodynamic behavior becomes increasingly important at temperature ranges above the physiological range due to the first-order phase transition from collagen to gelatin. This force detecting device can successfully detect and follow the force increase accompanying the phase transition in older animals 200gms and above but it is not sensitive enough to detect the phase transition in young animals with less crosslinked collagen. This equipment is being made more sensitive by the use of a more sensitive transducer. Under the conditions used, the heterogenetic phase transition was found to begin above 57°C and reach a maximum from 61°C to 70°C using this instrument depending on the amount of water in the skin which will greatly affect where the transition occurs. From other thermodynamic equipment it has been found the more edemic the skin, the lower the phase transition. It has been found that the beginning of the phase transition can be lowered into the forty degree range depending on the fluid content of the skin (which is a dilutant of the collagen in the skin).

Since, in the physiological temperature range, the dominant phenomena are hydrodynamics in nature further elongation detecting equipment must be designed to separate these phenomena and characterize them using kinetic theory. Once this has been done these various phenomena and their related parameters may be evaluated as a function of the biological variables of age and whole animal stress.